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Expression of an *Arabidopsis* phosphoglycerate mutase homologue is localized to apical meristems, regulated by hormones, and induced by sedentary plant-parasitic nematodes

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Abstract

We previously isolated a partial soybean cDNA clone whose transcript abundance is increased upon infection by the sedentary, endoparasitic soybean cyst nematode *Heterodera glycines*. We now isolated the corresponding full-length cDNA and determined that the predicted gene product was similar to the group of cofactor-dependent phosphoglycerate mutase/bisphosphoglycerate mutase enzymes (PGM/bPGM; EC 5.4.2.1/5.4.2.4). We designated the corresponding soybean gene *GmPGM*. PGM and bPGM are key catalysts of glycolysis that have been well characterized in animals but not plants. Using the *GmPGM* cDNA sequence, we identified a homologous *Arabidopsis thaliana* gene, which we designated *AtPGM*. Histochemical GUS analyses of transgenic *Arabidopsis* plants containing the *AtPGM* promoter*::GUS* construct revealed that the *AtPGM* promoter directs GUS expression in uninfected plants only to the shoot and root apical meristems. In infected plants, GUS staining also is evident in the nematode feeding structures induced by the cyst nematode *Heterodera schachtii* and by the root-knot nematode *Meloidogyne incognita*. Furthermore, we discovered that the *AtPGM* promoter was down-regulated by abscisic acid and hydroxyurea, whereas it was induced by sucrose, oryzalin, and auxin, thereby revealing expression characteristics typical of genes with roles in meristematic cells. Assessment of the auxin-inducible *AUX1* gene promoter (a gene coding for a polar auxin transport protein) similarly revealed feeding cell and meristem expression, suggesting that auxin may be responsible for the observed tissue specificity of the *AtPGM* promoter. These results provide first insight into the possible roles of PGM/bPGM in plant physiology and in plant-pathogen interactions.

Abbreviations: ABA, abscisic acid; bPGM, bisphosphoglycerate mutase; C_t, threshold cycles; dai, days after inoculation; GUS, β-glucuronidase; IAA, 3-indoleacetic acid; PCR, polymerase chain reaction; PGM, phosphoglycerate mutase; PGM-d, phosphoglycerate mutase cofactor-dependent; PGM-i, phosphoglycerate mutase cofactor-independent; qRT-PCR, quantitative real-time reverse transcription-PCR

Introduction

Plant-parasitic nematodes are among the most serious pests in world agriculture. Two of the most economically damaging groups are the cyst nematodes (*Heterodera* and *Globodera* spp.) and the root-knot nematodes (*Meloidogyne* spp.), which are responsible for major crop losses worldwide (Sasser and Freckman, 1987). For example, the soybean cyst nematode, *Heterodera glycines*, is the most damaging pathogen of soybean causing annual losses of several hundred million dollars in the USA alone (Wrather *et al.*, 2001a, b). Both groups (cyst and root-knot nematodes)

Nucleotide sequence data reported are available in the GenBank/EMBL/DDBJ databases under the accession number AY004240.

are sedentary biotrophic endoparasites of roots that induce and maintain complex feeding structures containing specialized feeding cells. In this interaction, infective second-stage nematode juveniles (J2s) enter host roots and migrate toward the vascular cylinder in search of suitable cells that can serve as initial feeding cells. J2s then initiate localized reorganization of the host cells' morphology and physiology, resulting in the formation of specialized feeding cells. Depending on the nematode genus, these cells either develop into a syncytia by fusion of neighboring cells (for cyst nematodes) or several cells are stimulated to form a system of discrete giant-cells that are embedded in a gall (for the root-knot nematodes) (Jones, 1981). Nematodes feed exclusively from their feeding cells as they complete their life cycles.

The plant hormone auxin plays a key role in a wide variety of growth and developmental processes. At the cellular level, auxin acts as a signal for cell division, extension, and differentiation during the course of plant development (Davis, 1995). Several studies have shown that auxin plays a role in feeding cell induction by cyst and root-knot nematodes, suggesting that auxin signaling is essential in both syncytium and giant-cell formation (reviewed by Goverse *et al.*, 2000a; Bird and Kaloshian, 2003). Consistent with this idea, it has been proposed that nematodes induce a local accumulation of auxin by controlling auxin distribution in the roots, likely by altering auxin efflux (Hutangura *et al.*, 1999; Goverse *et al.*, 2000b). Additionally, a recent study has suggested that after an initial auxin increase in the developing giant-cells, root-knot nematodes lower auxin biosynthesis in feeding cells at later stages of the infection process to alter plant cell development (Doyle and Lambert, 2003).

Feeding cell formation and maintenance are mediated through nematode signaling and accompanied by changes in plant gene expression (reviewed by Davis *et al.*, 2000; Hussey *et al.*, 2002; Williamson and Gleason, 2003). Several studies have shown altered plant gene expression in nematode pathosystems (reviewed by Gheysen and Fenoll, 2002; Favery *et al.*, 2002; Green *et al.*, 2002; Mazarei *et al.*, 2002; Juergensen *et al.*, 2003; Puthoff *et al.*, 2003; Thurau *et al.*, 2003). Identification and characterization of host genes that are potentially involved in the plant-nematode interaction, as indicated by nematode-induced changes in expression, might prove to be an important tool to aid in the understanding of the molecular mechanisms involved in nematode-plant interactions.

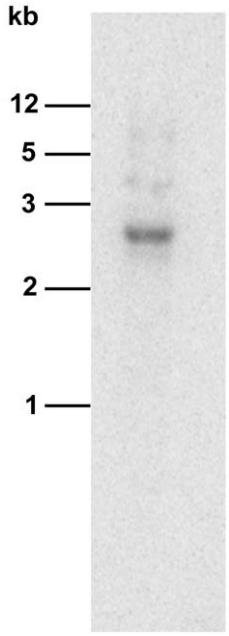

Figure 1. Southern blot of soybean genomic DNA probed with the soybean full-length D10.1 cDNA.Soybean genomic DNA (10 μg/lane) was digested with *Eco*RI and electrophoresed through a 1% agarose gel. The DNA blot was hybridized with the soybean full-length D10.1 cDNA. Numbers at left are molecular length markers in kb.

We previously used differential display of mRNA to isolate soybean cDNA clones corresponding to mRNA species that change abundance during the early stages of the compatible interaction between soybean and the soybean cyst nematode (*H. glycines*) (Hermsmeier *et al.*, 1998). In these studies, we showed that transcripts corresponding to a partial cDNA clone (D10.1) increased in *H. glycines*-infected roots of susceptible soybean one day post inoculation. Here we report a further characterization of this gene, including the isolation of a full-length cDNA and detailed analyses of its expression in soybean and *Arabidopsis* under a variety of conditions.

Materials and methods

Soybean and nematode cultivation

The soybean cultivar Corsoy 79 was used throughout this study. Corsoy 79 is susceptible to all *Heterodera glycines* biotypes tested (Bernard and Cremeens, 1988). Sterile soybean plants were established by germination of surface-sterilized seeds. Seeds were surface-sterilized by immersion in 70% ethanol for

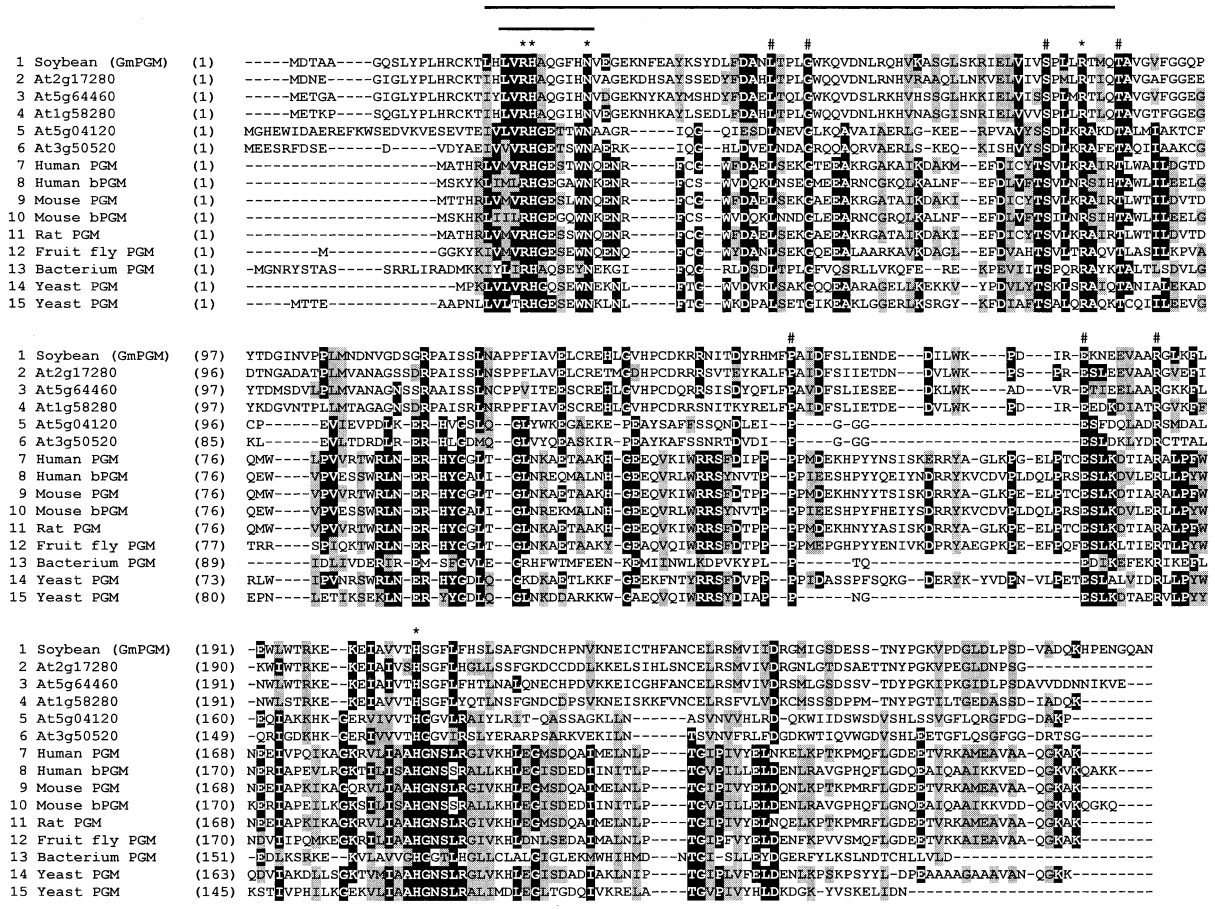

Figure 2. Alignment of the soybean and *Arabidopsis thaliana* phosphoglycerate mutase (PGM)-like proteins with PGMs from other organisms. The sources of sequence data are as follows: 1, soybean GmPGM (this study, GenBank accession number AY004240); 2–6, *A. thaliana* (Arabidopsis Genome Initiative At2g17280, At5g64460, At1g58280, At5g04120, At3g50520); 7, human PGM (GenBank P15259); 8, human bPGM (bisphosphoglycerate mutase) (GenBank P07738); 9, mouse PGM (GenBank AAC13263); 10, mouse bPGM (GenBank P15327); 11, rat PGM (GenBank P16290); 12, fruit fly (*Drosophila melanogaster*) PGM (GenBank S50326); 13, bacterium (*Aquifex aeolicus*) PGM (GenBank AAC07594); 14, yeast (*Saccharomyces cerevisiae*) PGM (GenBank PMBYY); 15, yeast (*Schizosaccharomyces pombe*) PGM (GenBank S43214). Sequences were aligned with the CLUSTAL-W program (Thompson *et al.*, 1994). Dashes indicate gaps in the amino acid sequences used to optimize the alignment. Regions of identical (black) or similar amino acid (shaded) present in at least 50% of the aligned sequences are indicated. The two regions selected as PGM signature patterns (Inter Protein IPR001345: Prosite PS00175 and Protein Family PF00300) are indicated by solid lines. Conserved residues that constitute the active sites are marked by asterisk (*). Strictly conserved residues among PGMs are indicated by #.

5 min and 2.1% sodium hypochlorite for 12 min, and then rinsed three times in sterile distilled water for 10 min. Seeds were germinated in the dark at 26 °C on 1% water-agar for two days, transferred to magenta boxes (Sigma, St. Louis, MO) containing sterile sand supplemented with Hoagland growth solution (Sigma), and grown at 26 °C with a 16 h photoperiod of ca. 2400 lx provided by fluorescent light bulbs.

The *H. glycines* inbred line OP50 (Dong and Opperman, 1997) was propagated in greenhouse cultures with Corsoy 79 soybeans as hosts. Inoculated soybean roots were examined for developmental stages of the nematodes by staining with acid fuchsin following the procedure of Hussey (1990). Stained nematodes were examined with the dissecting microscope.

Soybean inoculation and tissue harvesting

Nematodes for soybean root inoculation were surface-sterilized for 12 min in 0.01% $HgCl_2$ and then washed three times in sterile distilled water. Nematodes were pelleted and then re-suspended in 1.5% low-melting-point agarose (Life Technologies, Gaithersburg, MD)

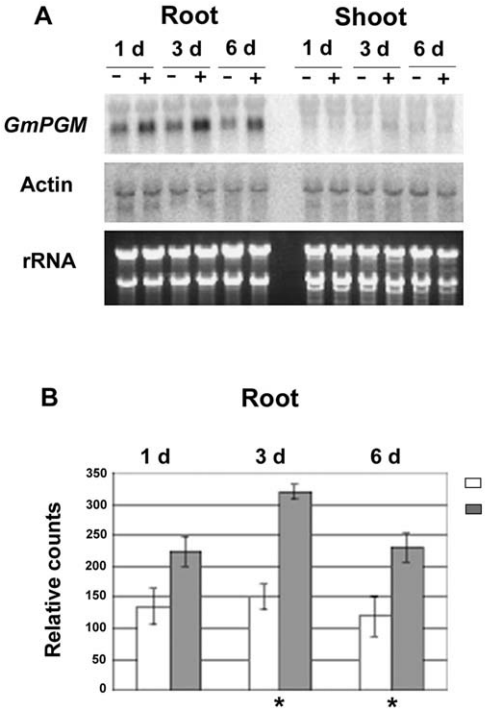

Figure 3. RNA blot analysis of *GmPGM*. A. *GmPGM* mRNA accumulation in nematode-infected and uninfected plants. Blots of total RNA (10 μg/lane) were obtained from roots and shoots of *Heterodera glycines*-infected (+) and uninfected (−) soybean plants at 1, 3, and 6 days (d) after nematode inoculation. Blots were standardized with a soybean actin probe (Actin) (Nagao *et al.*, 1981), which we have previously shown to be constitutively expressed during the early *H. glycines* infection stages (Hermsmeier *et al.*, 1998). The ethidium bromide stain of the agarose gel was used to verify equal RNA loading before blotting (rRNA). Autoradiographs were recorded after three days of exposure to a phosphorimager screen. Representative blots are shown. Similar results were obtained in three other experiments. B. Quantification of *GmPGM* expression. Intensity of the hybridization signals detected in the soybean roots was measured with a phosphorimager and quantified with the ImageQuant software. *GmPGM* hyrbidization intensity values were adjusted relative to the constitutive actin hybridization intensity values (relative counts). Each bar represents the mean of four independent northern blots with the standard errors of the noted mean. Significance of the induction of *GmPGM* expression at each time point (infected compared with uninfected) was determined statistically by a two-sample *t*-test (P <0.05). Asterisks indicate significant differences.

at 37 °C. Plants were inoculated at the two-true-leaf stage after about eight days of growth in sand-filled magenta boxes. Inoculation was performed by applying 1 ml of an agarose-nematode suspension, containing about 1000 nematodes, to plant rooting zones via narrow holes created in the sand substrate prior to inoculation. Control plants were treated with agarose in the absence of nematodes. Plants were allowed

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-1130                ATCAACAATAAAACCAGGAACTCAAAACATACGGTCATATAAGAT
-1085  TCTCTTGCAAGTAGATACGAGGCGTTGTGTAGCGATTAGAAGGAGCTTTTTGATATTTTC
-1025  GTTTCATGCGCAAATGCATTTGTCTTGAACCCCTGCCTGAAATTGGATTGTAAGTAAAGT
-965   GCTAATGATGGATATTGCTGTAAACAGAAACAATGATCTTTAACTGATTGATGTCAAAGG
-905   AATGAGTCTGGTTTTATCTTTGGTATGCTCTGAACAATTTTTGTATGAGAAGAGAAGACT
-845   GAGATTTGCTGTTTTTAGATTGTTCAATAGCTTCCAAGTACTTTGGTTTTAGGCTTTTAG
-785   CTAATTAGTTTGTGCCGTCTTCATATTTTCCCCTTCTTCTTTGCAATTTTGCTATTTTAT
-725   GGAAGCTGATTATGACCAAGCATCAATGAAGGAATCTCTCCACTATAACTTATCAGGTTC
-665   ACATAACTTTCTTAAAAGTAAAACAGTGAAGAACCATTCACCTTCCATATCTCCAGAGCA
-605   GGTTCATTGCATCTTAGTTAGGGTTTAACCTTCAAACTTGTCTTTACACTATCCTAGGAT
-545   TCTATTGTTTTCTTGTCTTCTTTGTATTGCTGCATTTGATTGACTTACCACTACTCTCTT
-485   GAGATTGGCCAATTTTTGCTGATTAACAGTTCATTTTTGCTCTAAGTTTCATCAGTTTTT
-425   TTTCTTGGCATGGATGTTTTGCTGAATTATCATTCGCCTAATGAGTTACACAAACAAAAT
-365   TGTAGATTTTCAGAGAAAACAAAACAAAACATATGAATAAAAAAGTTATGCATATGATAC
-305   GCGTTAAGAAAAACAAATGATTAACGTACGTCGGCTGTGAAGTGTTGTTTGATACAATTG
-245   GGGGAAGTGACGGCACAATGGGCTGGTTTCACGGCCCCTTTCTAAGTCATACACGTCTCC
-185   TTTTTTTATTTTCGTTTTTTTCAAAATAATAAAAATAATATAGAGAGTGGCATATCCGAC
-125   GGTTTAGGATCTCTGCCTTTTCTCAACTTATAGTATATACCAAACACTTTCTAAGGTAAC
-65    ATGAGATATTTCACATCCTTTTCTCTCTTATTCGTTTCTGAGATTCCCTTCTTGTTGTTG
-5     TAGCCATG
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Figure 4. Sequence of the *AtPGM* 5′ promoter fragment. Nucleotide sequence of the promoter fragment directly upstream of the *AtPGM* (Arabidopsis Genome Initiative At2g17280) coding region. The nucleotides corresponding to the primers used for amplifying the *AtPGM* promoter fragment are marked by boxes. The translation initiation codon is indicated by asterisks. Putative *cis*-acting elements were determined by the PLACE database (Hingo *et al.*, 1998). Auxin-responsive elements (GmSAUR) (Xu *et al.*, 1997) are indicated by single underline; the abscisic acid-responsive element (ABRE) (Busk and Pagès, 1998) is indicated by double underline; the nematode box (Escobar *et al.*, 1999) is indicated by triple underline; the mitosis-specific activator-like sequence (MSA) (Ito *et al.*, 1998) is marked by an arrowed underline; and the sugar-response element (amy3Os) (Hwang *et al.*, 1998) is marked by a dashed underline.

to continue growth as described above. At different time points after inoculation, plants were taken out of the sand, and their roots were washed by rinsing with sterile distilled water. Root and shoot (including cotyledons and two-true-leaf) tissues of eight plants were harvested at each time point, frozen in liquid nitrogen, and stored at −80 °C until use. To ensure aseptic conditions, the agarose-nematode suspension used for plant inoculation was also applied to sterile soybean plants established on Gamborg's B5 medium (Life Technologies). These cultures were monitored for signs of microbial contamination. An inoculation series that remained clear of microbial contamination was used for the remainder of this work.

Soybean cDNA library screening

A Uni-ZAP XR directionally cloned, oligo-dT-primed cDNA library was constructed (Stratagene, La Jolla, CA) with RNA prepared from infected Corsoy 79 soybean roots harvested at 1, 2, and 3 days after inoculation with the *H. glycines* inbred line OP50. cDNA clone D10.1 was used to screen the non-amplified library fraction. The probe was radiolabeled by PCR of gel-purified cDNA fragment with vector-specific primers pGEM-up and pGEM-down (Herms-

meier *et al.*, 1998). About 350 000 recombinant bacteriophage from the cDNA library were screened by hybridization with radioactively labeled D10.1 clone. Positive plaques were isolated, purified, and excised *in vivo* following the manufacturer's instructions.

DNA sequencing

Recombinant plasmids isolated from the soybean root cDNA library were purified with the Qiagen Plasmid Mini Kit (Qiagen, Chatsworth, CA) and subjected to sequencing at the Iowa State University DNA Sequencing and Synthesis Facility (Ames, IA). Sequence analysis was performed with the BLAST (Altschul *et al.*, 1997) and DNASIS (Hitachi Software Engineering, San Bruno, CA) programs.

RNA and DNA gel blot analyses

For RNA blot analyses, total RNA was isolated from frozen root and shoot tissues of eight *H. glycines*-infected and uninfected soybean plants each ground under liquid nitrogen as described by Pawlowski *et al.* (1994). RNA (10 μg) from each sample was separated on 1% formaldehyde agarose gels and transferred onto nylon membranes (S&S Nytran Plus, Schleicher & Schuell, Keene, NH) by a wet blotting procedure (Sambrook *et al.*, 1989). RNA was fixed to the membranes in a FB-UVXL-1000 cross-linker (Fisher Scientific, Pittsburgh, PA). Four independent blots were prepared from the total RNA.

GmPGM insert in the pBluescript SK(−) vector obtained from the cDNA library screen was amplified by PCR with vector-specific primers T3 and T7 (Integrated DNA Technology, Coralville, IA). PCR product was gel-purified with a Qiaex II Kit (Qiagen), radiolabeled via PCR, and then used as a probe in soybean RNA blot analyses. Hybridizations were carried out at 42 °C in a hybridization buffer composed of 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50% formamide, 0.1% sodium dodecyl sulfate (SDS), 5× Denhardt's solution (Sambrook *et al.*, 1989), 0.1 mg ml^{-1} herring-sperm DNA, and 3 × 10^6 cpm/ml of labeled gene probe. The hybridized blots were washed three times for 15 min in 0.1× SSC/0.1% SDS at 65 °C. Bound radiolabeled probes were imaged with a Molecular Dynamics Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, CA), which allowed quantification of hybridized probe by using ImageQuant software (Molecular Dynamics).

A blot of *Eco*RI-digested soybean genomic DNA was prepared as described (Hermsmeier *et al.*, 1998). DNA gel blot hybridization and analysis was performed as explained above.

Isolation of AtPGM *promoter region from* A. thaliana

Sequencing and annotation of the BAC clone F5J6 from chromosome 2 which contains the *AtPGM* gene (GenBank accession number AC002329/F5J6.4) revealed that 1155 bp upstream of the *AtPGM* coding region lies another open reading frame (F5J6.3). Therefore, the *AtPGM* promoter is likely positioned entirely within this intergenic region and the 1155 bp genomic fragment directly upstream of the *AtPGM* coding region contains all the regulatory elements required to direct correct temporal and spatial expression of the *AtPGM* gene. Due to the lack of information concerning the exact length of the *AtPGM* promoter, we PCR-amplified the 1130 bp genomic fragment upstream of the *AtPGM* coding sequence (see below) and used it in the promoter analyses described in this study.

Promoter construct

The *AtPGM* promoter was isolated by PCR amplification from *A. thaliana* genomic DNA prepared as described by Rogers and Bendich (1994). Primers were designed to amplify the *AtPGM* promoter (corresponding to 1130 bp upstream of the translation start site) and to create *Sal*I (at the 5′ end) and *Bam*HI (at the 3′ end) restriction sites. The primers used were: AtPGM-P forward, 5′-AT*GTCGAC*ATCAACAATAAAACCAGGAACT-3′ and AtPGM-P reverse, 5′-AT*GGATCC*GGCTACAAC AACAAGAAGGGAA-3′. PCR amplification was performed with *Taq* polymerase (Life Technologies) in a three-temperature program (94 °C, 55 °C, and 72 °C for 1 min each) with 30 cycles after 5 min of incubation at 94 °C. The PCR product was digested with *Sal*I and *Bam*HI, gel-purified, and ligated into binary vector pBI101.1 (Jefferson *et al.*, 1987). The binary construct was maintained in *Escherichia coli* strain DH5α. Both strands of the promoter construct were sequenced to verify wild-type sequences.

Transgenic Arabidopsis thaliana *plants, nematode infection, and treatments*

Agrobacterium tumefacienes strain C58 was transformed with the *AtPGM::GUS* construct by the freeze-

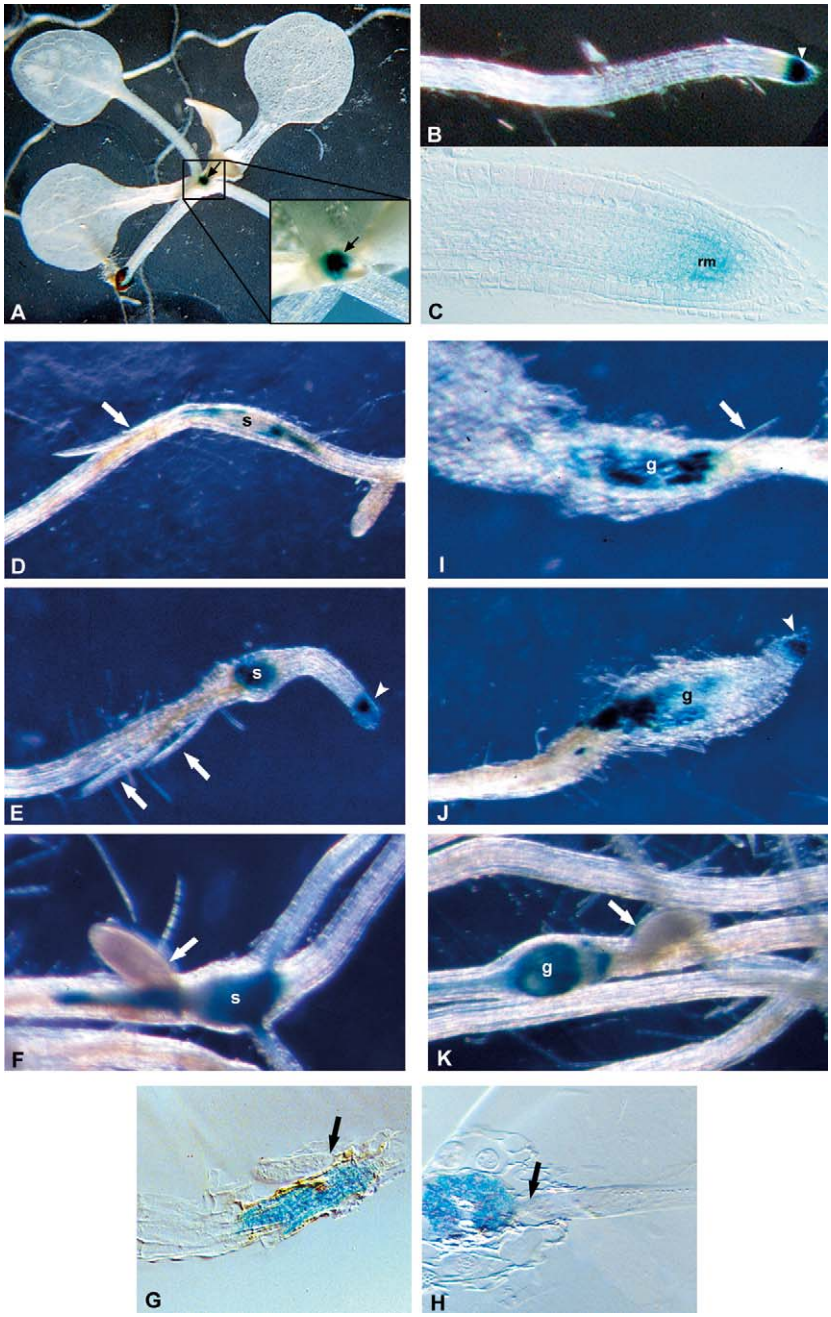

Figure 5. Histochemical staining for GUS activity in transgenic *A. thaliana* plants containing the *AtPGM::GUS* construct. GUS expression in shoot (A) and in root (B, C) of a 12-day old *A. thaliana* plant. The inset in A and the arrow indicate GUS activity in the shoot apical meristem. The arrowhead in B indicates GUS activity in the root tip. A root longitudinal section (C) shows GUS activity in the root apical meristem (rm). More than 10 independent transformed lines were tested for GUS expression and similar observations were obtained. D–H. GUS expression in transgenic *A. thaliana* plants infected with cyst nematode *Heterodera schachtii*. Localized GUS expression in syncytium (s) at 3 days after inoculation (dai) (D), at 7 dai (E), and at 14 dai (F). A root longitudinal section (G) and a cross section (H) reveal the anterior ends of sedentary nematodes (arrows) associated with feeding cells showing strong GUS activity at 3 dai. I–K. GUS expression in transgenic *A. thaliana* plants infected with root-knot nematode *Meloidogyne incognita*. Localized GUS expression within the nematode-induced galls (g) containing the giant-cells at 3 dai (I), at 7 dai (J), and at 14 dai (K). Arrows indicate nematodes. Arrowheads indicate GUS expression in root tips, which also is observed in uninfected roots. The differences in GUS expression between nematode-infected and uninfected roots were observed in 5 independent transformed lines for which at least 10 seedlings each were assayed.

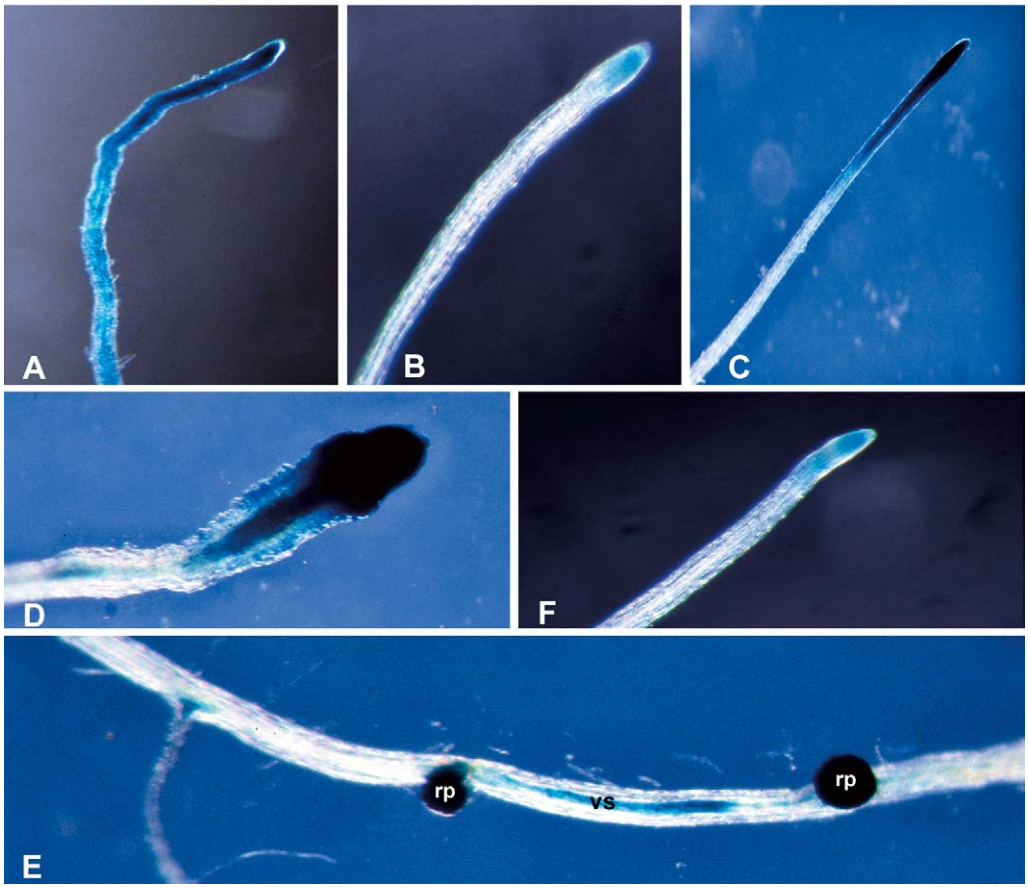

Figure 6. Promoter activity of *AtPGM::GUS* in transgenic *A. thaliana* plants subjected to various treatments. Two-week old transgenic plants were grown for 48 h on medium supplemented with each treatment. At least 10 seedlings from 5 independent transgenic lines were used for each assay and typical examples of GUS staining are shown. A typical example of untreated control plants is shown in Figure 5B. Histochemical GUS expression in roots of *A. thaliana* plants treated with 50 μM 3-indoleacetic acid (A), 100 μM abscisic acid (B), 6% sucrose (C), 30 μM oryzalin (D and E), or 100 mM hydroxyurea (F). rp, lateral root primordium; vs, vascular cylinder.

thaw method (An *et al.*, 1988), and used to generate transgenic *A. thaliana* plants. The transformed *A. tumefacienes* was grown in LB (Difco, Detroit, MI) medium containing 50 mg/ml gentamycin and kanamycin (Fisher, Fair Lawn, NJ). *A. thaliana* (wild-type Columbia) transformation was performed as previously described (Clough and Bent, 1998). At least 15 independent transgenic *A. thaliana* plants were generated for the promoter construct.

For nematode experiments, *Heterodera schachtii* and *Meloidogyne incognita* were propagated in greenhouse cultures with sugar beet (cv. Monohi) and tomato (cv. Rutgers), respectively, as hosts. Surface-sterilized J2 of the nematodes were prepared as previously described (Baum *et al.*, 2000). Transgenic *A. thaliana* seeds (T_2 generation) were surface-sterilized with 2.6% sodium hypochlorite for 5 min, washed three times with sterile distilled water, then planted aseptically, one seed per well, in 12-well culture plates containing modified Knop medium (Sijmons *et al.*, 1991) solidified with 0.8% Daishin agar (Brunschwig Chemie, Amsterdam, Netherlands). Plants were grown at 26 °C with a 12-h photoperiod of ca. 1500 lx provided by fluorescent light bulbs. Transgenic plants 10–12 days old were inoculated individually with ca. 500 surface-sterilized J2. Four plants in the 12-well plate were arbitrarily positioned as non-inoculated control plants. The plants were maintained in the temperature and lighting regimes mentioned above. Histochemical GUS analyses were performed (see below) to examine the GUS activity.

For experiments, transgenic plants were grown on Knop medium for two weeks under the same temperature and lighting regimes mentioned above. Wounding

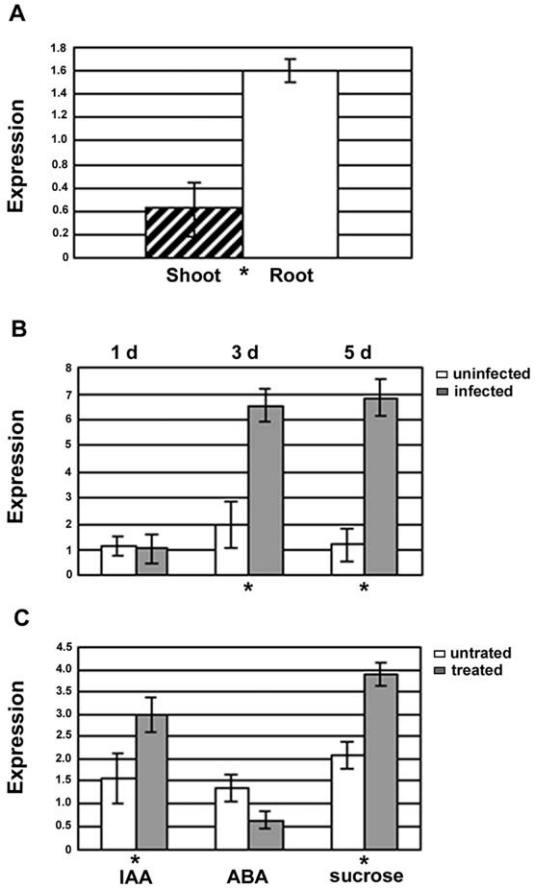

Figure 7. Quantitative real-time RT-PCR (qRT-PCR) analysis of *AtPGM* mRNA steady-state levels. Total RNA was isolated from respective tissues of *A. thaliana* plants. First-strand cDNA was synthesized and subjected to qRT-PCR in triplicate on the iCycler as described in Materials and methods. Relative *AtPGM* mRNA quantities (Expression) were determined with iCycler software as detailed in Materials and methods. Each bar represents the mean of three independent experiments with the standard errors of the noted mean. Significant *AtPGM* mRNA expression changes were determined statistically by a two-sample *t*-test ($P < 0.05$). Asterisks indicate significant mRNA changes. A. *AtPGM* mRNA accumulation in shoots and roots of 2-week old *A. thaliana* plants. B. *AtPGM* mRNA accumulation in *H. schachtii*-infected and uninfected roots of 12-day old *A. thaliana* plants at 1, 3, and 5 days (d) after nematode inoculation. C. *AtPGM* mRNA accumulation in roots of 2-week old *A. thaliana* plants in the absence (untreated) and presence (treated) of 50 μM 3-indoleacetic acid (IAA), 100 μM abscisic acid (ABA), or 6% sucrose for 48 h.

was performed by repeatedly piercing the roots and leaves of the plants with sterile pipette tips. At 10 h, 3 days, or 7 days, wounded plants were removed from the Knop plates and stained for GUS activity (see below). Control plants were maintained under the same conditions in the absence of wounding. For IAA, ABA, and sucrose treatments, plants were harvested from Knop plates and were then incubated in Knop medium without or with, respectively, 50 μM 3-indoleacetic acid (3-IAA, Sigma), 100 μM abscisic acid (ABA, Sigma), and 6% sucrose (Fisher) for 48 h. For hydroxyurea and oryzalin treatments, plants were harvested from Knop plates and then transferred to the same medium containing 100 mM hydroxyurea (Sigma) or 30 μM oryzalin (Sigma), and were kept for 48 h. Control plants were kept under the same conditions in the absence of hydroxyurea or oryzalin treatments. The treated and untreated plants were stained with GUS staining solution in histochemical GUS analysis (see below), or were harvested, frozen in liquid nitrogen, and kept at -80 °C until use in fluorometric GUS analysis (see below).

Histochemical GUS analysis

Histochemical assays for GUS expression were performed with the substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) (Research Products International, Mt. Prospect, IL) according to standard protocols (Jefferson, 1987). GUS staining was carried out overnight at 37 °C.

For the nematode experiments, GUS activity was determined in transgenic *A. thaliana* plants by adding GUS staining solution to each well, without disturbing the roots. Nematode-infected and uninfected plants were stained for GUS activity at varying times after nematode inoculation. After GUS staining, tissues were either cleared by replacing the GUS solution with 70% ethanol, without disturbing the plant or prepared for further cytological analyses, as described below. GUS activity was monitored microscopically in shoot and root tissues.

For the treatment experiments, the treated and untreated plants were incubated in the GUS staining solution overnight at 37 °C. The stained plants were cleared with 70% ethanol and then examined for GUS activity microscopically.

Stained tissues were observed under a Zeiss Stemi SV 11 dissecting microscope and images were recorded onto Kodak Ektachrome 64T tungsten slide film. Slides were scanned into Adobe Photoshop 6.0, adjusted for brightness and contrast, and assembled into figures.

For cytological studies, GUS-stained *A. thaliana* plants were carefully removed from their medium and fixed in a mixture of freshly prepared 2% glutaraldehyde (Ted Pella, Redding, CA) and 2% formaldehyde (Fisher) in 50 mM PIPES (1,4-piperazinediethane

sulfonic acid; Fisher) plus 10% sucrose pH 7.2 under vacuum overnight at 4 °C. Samples were washed in 50 mM PIPES pH 7.2, dehydrated in a graded ethanol series to 95% ethanol and infiltrated and embedded in glycol methacrylate resin (Electron Microscopy Sciences, Fort Washington, PA). Samples were sectioned on a Reichert Ultracut S ultramicrotome (Leica Microsystems, Wetzlar, Germany) at 3–5 μm, mounted on glass slides and examined on an Olympus BH-2 compound microscope equipped with differential interference contrast (DIC) optics. Images were recorded and figures were assembled as described above.

Fluorometric GUS analysis

Fluorescence of 4-methylumbelliferone (4-MU) produced by cleavage of 4-methylumbelliferyl-β-D-glucuronide (MUG) (Research Products International) was measured to quantify GUS activity according to standard protocols described by Jefferson (1987). Protein concentration was determined with a Pierce BCA-200 Protein Assay Kit (Pierce, Rockford, IL).

AtPGM *mRNA expression analyses*

Quantitative real-time reverse transcription-PCR (qRT-PCR) (Bustin, 2002) was used to quantify *AtPGM* mRNA abundance changes in *A. thaliana* shoots and roots in a variety of treatments.

For nematode treatment, *A. thaliana* plants (wild-type Columbia) were grown under *in vitro* conditions and inoculated with *H. schachtii* as described above. At varying time points after inoculation, root and shoot tissues of 10 plants per treatment (inoculated and non-inoculated control) were harvested, frozen in liquid nitrogen, and stored at −80 °C until use.

For hormone and sucrose treatments, *A. thaliana* plants were grown in soil (Sunshine Professional Growing Mix, Bellevue, WA) at 26 °C with a 12 h photoperiod of ca. 1500 lx provided by fluorescent light bulbs. Treatments were performed on 2-week old plants. The root systems of whole plants were washed gently with water to remove soil and then the plants were used for each treatment. Ten plants per treatment were used. The whole plants were incubated for 48 h in either 50 μM IAA, 100 μM ABA, or 6% sucrose. Control plants were incubated in water alone. Root and shoot tissues were harvested, frozen in liquid nitrogen, and kept at −80 °C until use.

In order to conduct qRT-PCR assays for *AtPGM*, the following gene-specific primers to the 3′ region were designed: qAtPGM forward, 5′-CACGGTCTCT TGAGTTCTTTCGGGAAGG-3′, and qAtPGM reverse, 5′-CTCGATCCACGATAACCATTGAGCGA-3′. These primers amplified a single product of 109 bp, as confirmed by the melting temperature of the amplicons and gel electrophoresis. Total RNA was isolated from the respective frozen tissues on RNeasy columns (Qiagen, Valencia, CA) following the manufacturer's instructions. As described in Puthoff *et al.* (2003), 5 μg of total RNA was denatured in the presence of an oligo-d(T) primer, cooled on ice and divided evenly into three reverse transcription reactions. One of these reactions was monitored for cDNA synthesis with ^{32}P-dCTP (NEN, Boston, MA) incorporation, a second reaction was used as the RT control (no reverse transcriptase was added), and the third reaction was used for later qRT-PCR, which was conducted in triplicate. The following additions were made to each reaction: 4 μl 5× first-strand buffer (Gibco-BRL, Rockville, MD), 2 μl 0.1 M DTT, 1 μl 10 mM dNTP mix, 1 μl Superscript II Reverse Transcriptase (Gibco-BRL). In the RT control reaction water was substituted for Superscript II. Each 20 μl reaction was incubated at 42 °C for 1 h. With the ^{32}P-monitored reaction as a guide, first-strand cDNA was diluted to ensure an equal amount of cDNA (equivalent to 10 ng of total RNA/PCR reaction) was placed in each PCR reaction. PCR was conducted on the iCycler (BioRad, Hercules, CA) with a 96-well reaction block in the presence of SYBR Green under the following conditions: 1× PCR buffer (Invitrogen, Carlsbad, CA), 3 mM $MgCl_2$, 0.4 mM dNTP, 0.1 μM each primer, 10 nM fluorescein (BioRad, Hercules, CA), 1:100 000 dilution SYBR Green, 10 000 × stock, 1 U Platinum *Taq* (Invitrogen, Carlsbad, CA) in a 25 μl reaction. PCR cycling was 95 °C for 3 min, followed by 45 cycles of 15 s at 95 °C, 30 s at 60 °C. Threshold cycles (C_t) were determined with iCycler (BioRad) software for all treatments. To quantify relative mRNA concentrations, a standard curve was prepared for each 96-well plate PCR reaction. For this purpose, a threefold dilution series of a total of six dilutions was prepared from an *A. thaliana* root RNA sample (which was known to contain detectable *AtPGM* mRNA amounts), and each dilution was subjected to qRT-PCR analyses in triplicate with the *AtPGM*-specific primers. Obtained C_t values were used by the iCycler software package to plot a standard curve that allowed quantification of *AtPGM* mRNA in other RNA samples relative to the RNA sample used to prepare the standard curve. These relative mRNA quantities of RNA samples are presented as 'Expression' values in Figure 7 and allowed the

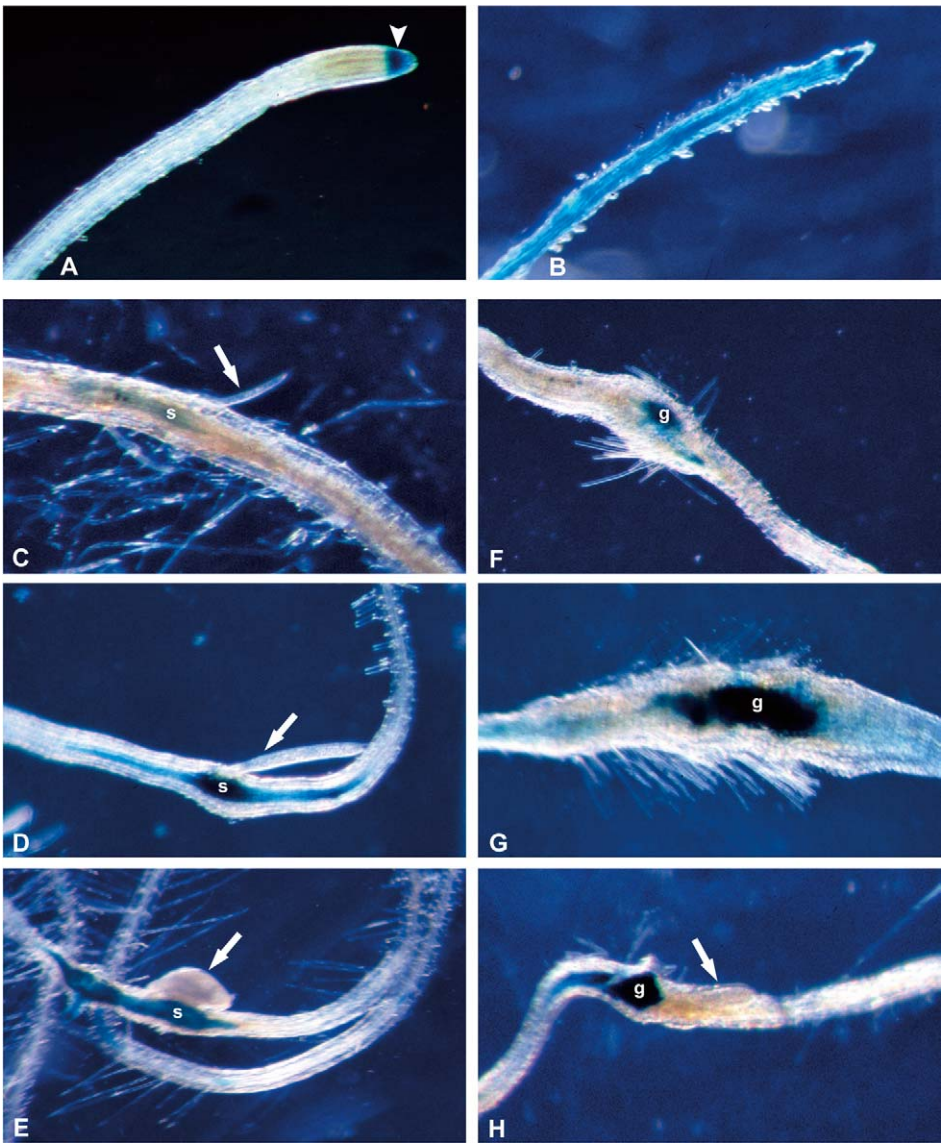

Figure 8. Promoter activity of *AUX1::GUS* in transgenic *A. thaliana* plants. Histochemical GUS expression in roots of *A. thaliana* plants untreated (A) and treated with 50 μM 3-indoleacetic acid for 48 h (B). The arrowhead (A) indicates GUS activity in the root tip. C–E. GUS expression in transgenic *A. thaliana* plants infected with cyst nematode *Heterodera schachtii*. Localized GUS expression in syncytium (s) induced by *H. schachtii* 3 days after inoculation (dai) (C), 7 dai (D), and 14 dai (E). F–H. GUS expression in transgenic *A. thaliana* plants infected with root-knot nematode *Meloidogyne incognita*. Localized GUS expression in root galls (g) induced by *M. incognita* 3 dai (F), 7 dai (G), and 14 dai (H). Arrows indicate nematodes.

comparison of relative mRNA amounts among treatments. For quality assurance purposes, only qRT-PCR assays that resulted in standard curves with the following parameters (Bustin, 2002), as calculated by the iCycler software, were considered: (1) linear standard curve throughout the measured area, (2) standard curve slope between −3.5 and −3.2, and (3) R^2 above 0.99.

Results

Identification of a full-length D10.1 cDNA

Screening of a soybean cDNA library prepared from *H. glycines*-infected susceptible soybean roots with D10.1 led to the isolation of a 1150 bp cDNA sequence (GenBank accession number AY004240). This cDNA contained an open reading frame that was initiated by

an ATG, was terminated by a poly(A) tail, and corresponded in size to that determined by mRNA gel blot analysis (see below). Therefore, this cDNA can be considered full-length.

In DNA blot analyses of *Eco*RI-digested soybean genomic DNA, only one DNA fragment hybridized strongly when the blot was probed with the full-length D10.1 soybean cDNA (Figure 1). Therefore, it appears that only one copy of the gene and no other very closely related sequences are present in the soybean genome. This is confirmed by the fact that no other hybridizing cDNAs were identified during the library screen. In addition, searches of the soybean expressed sequence tag (EST) collection identified only two sequences (GenBank accession numbers AW102256 and BI315691) with homology to the D10.1 full-length cDNA. These were both identical to the D10.1 sequence suggesting they are from a single gene.

The nucleotide and deduced amino acid sequences of the D10.1 full-length cDNA were subjected to database comparisons using BLAST algorithms (Altschul *et al.*, 1997). These analyses revealed that the predicted protein belonged to the phosphoglycerate mutase (PGM) enzymes, specifically to the cofactor-dependent phosphoglycerate mutase/bisphosphoglycerate mutase (PGM/bPGM) group. The most conserved regions of these proteins are the PGM domain and the amino acid residues that constitute the active site (Figure 2). Because of the high similarity to PGM, we called the gene corresponding to the D10.1 sequence *GmPGM*.

Searches of the *Arabidopsis thaliana* genome sequence revealed five *A. thaliana* genes (Arabidopsis Genome Initiative (AGI) At2g17280, At5g64460, At1g58280, At5g04120, At3g50520) containing characteristic PGM/bPGM elements. Alignment of GmPGM and these *A. thaliana* PGM-like proteins along with PGM/bPGM homologues from other organisms is presented in Figure 2. Because of the highest similarity (72% identity, 85% similarity) of GmPGM with the *A. thaliana* PGM protein encoded by At2g17280, we focused on this *A. thaliana* gene for further functional characterization and refer to the corresponding gene as *AtPGM*.

GmPGM *expression analyses*

Our previous studies (Hermsmeier *et al.*, 1998) assessed *GmPGM* mRNA expression in *H. glycines*-infected roots at 1 day after inoculation (dai). In this current report, we extended these studies by using blot hybridizations to assess mRNA levels in shoots and roots of healthy and infected soybean plants at 1, 3, and 6 dai. Representative blots are shown in Figure 3A. Similar results were obtained in three other experiments. *GmPGM* transcripts were barely, if at all, detectable in total RNA from shoot tissues but were readily detectable in roots (Figure 3A). We observed increased mRNA steady-state levels of *GmPGM* in roots after *H. glycines* infection throughout the time period assessed (Figure 3A and B). On the other hand, nematode infection had no influence on *GmPGM* mRNA abundance in shoots (data not shown).

AtPGM *expression analyses*

To advance our understanding of PGM function in plant physiology and during nematode infection, we chose the *AtPGM* gene for further study in *Arabidopsis*, which is readily infected by the sugar beet cyst nematode, *H. schachtii*, and the root-knot nematode, *Meloidogyne incognita*. For this purpose, we isolated the promoter of *AtPGM* by PCR amplification (Figure 4), and multiple lines of transgenic *A. thaliana* plants expressing an *AtPGM* promoter*::GUS* construct were generated. Histochemical assays of GUS expression were used to analyze the temporal and spatial characteristics of this promoter during plant development, nematode parasitism, and other stresses.

Unstressed plants.
Shoots of some *AtPGM::GUS* lines occasionally showed GUS expression in the apical meristem (Figure 5A). However, in roots of all *AtPGM::GUS* lines all root tips showed strong GUS expression (Figure 5B). These assays were continued up to 5-week old plants and no detectable expression in any other tissues was observed. Sections of root tips showed that the *AtPGM* promoter directs detectable GUS expression specifically to the meristematic zone of the root tips (Figure 5C) indicating that *AtPGM* is a meristem-specific gene.

Nematode-infected plants.
AtPGM::GUS A. thaliana plants were analyzed for GUS staining at 3, 7, 14, and 21 dai with *H. schachtii*. These analyses revealed a pronounced increase of GUS expression specifically localized to the area of cyst nematode-induced feeding structures (syncytia) by 3 dai (Figure 5D), i.e., at the very early stages of syncytium formation. GUS activity continued to be increased at 7 dai (Figure 5E) and 14 dai (Figure 5F).

By 21 dai, GUS activity no longer was detectable within syncytia (data not shown). The localization of GUS activity within the *H. schachtii*-induced syncytia also was demonstrated in sections of infected roots (Figure 5G, H). To determine whether the effects described above were specific for the *Arabidopsis-H. schachtii* interaction or would also occur in other sedentary nematode interactions, we assessed GUS expression in roots infected by the root-knot nematode *M. incognita*. Also with this nematode, strong GUS activity was found in the feeding structures (galls), which contain the giant-cells (Figure 5I–K). As an additional treatment, *AtPGM::GUS* transgenic plants were mechanically wounded and assayed for GUS expression at 10 h, 3 days, and 7 days after wounding. Mechanical wounding did not result in any changes in GUS expression (data not shown).

Plants treated with plant hormones, sucrose, or cell cycle inhibitors.

The *AtPGM* promoter directs GUS reporter gene expression specifically to the root and shoot apical meristems, which suggests a role in meristematic activity. Several studies have demonstrated varied effects of exogenously applied plant hormones, sugars, and cell cycle inhibitors on the expression of genes implicated in meristematic activity (Hemerly *et al.*, 1993; John *et al.*, 1993; Ferreira *et al.*, 1994a, b; Doerner *et al.*, 1996; Burssens *et al.*, 1998; Trehin *et al.*, 1998; Riou-khamlichi *et al.*, 2000; Himanen *et al.*, 2002). Therefore, we examined the effect of similar stimuli on GUS activity in *AtPGM::GUS* plants. *A. thaliana* plants were treated with auxin (IAA, 50 μM) and ABA (100 μM) and analyzed for changes in GUS expression. As shown in Figure 6A, transgenic plants responded to IAA with a clear increase of GUS activity when compared to untreated plants (a typical example is shown in Figure 5B). In the case of ABA treatment, transgenic plants showed decreased GUS expression in the root tips (Figure 6B) compared to untreated plants (Figure 5B). To assess the influence of a sugar on *AtPGM* expression, transgenic *A. thaliana* plants were incubated in a 6% sucrose-containing medium. As shown in Figure 6C (cf. Figure 5B), an enhanced GUS expression extending from the root tips to the root elongation zone was observed in the sucrose-treated roots. We used hydroxyurea (DNA synthesis blocker) (Young and Hodas, 1964) and oryzalin (mitosis blocker) (Sree Ramulu *et al.*, 1991) as cell cycle inhibitors. Figure 6D and E show that GUS activity is increased in oryzalin-treated root tips and lateral root primordia as well as in regions of the vascular cylinder. In contrast, a reduction in GUS activity was observed in hydroxyurea-treated roots (Figure 6F). These effects of the treatments mentioned above on *AtPGM::GUS* were confirmed by quantitative fluorometric GUS activity assays (data not shown). The fact that *AtPGM* is expressed in meristems and is regulated by stimuli known to affect the expression of genes that function in meristems and during the cell cycle strongly suggests that the AtPGM is closely associated with meristem function and cell cycle activity.

qRT-PCR confirmation of promoter-GUS results

To validate the promoter studies, we used qRT-PCR. In these assays we quantified *AtPGM* mRNA steady-state levels in roots and shoots of *A. thaliana* plants subjected to the various treatments described above. *AtPGM* mRNA accumulation was detected in *A. thaliana* shoots and roots, with higher levels present in the roots (Figure 7A). Furthermore, we observed altered mRNA steady-state levels of *AtPGM* after *H. schachtii* infection: mRNA abundance increased in infected roots when compared to uninfected roots (Figure 7B). We also examined the effects of IAA, ABA, and sucrose treatment on *AtPGM* mRNA steady-state levels. As shown in Figure 7C, *AtPGM* mRNA abundance increased after IAA and sucrose treatment, whereas it decreased after ABA treatment. All these measurements are in agreement with the GUS expression patterns. Therefore, we conclude that the promoter characteristics as observed by GUS staining more than likely reflect the actual temporal and spatial mRNA accumulation of the *AtPGM* gene.

Putative cis*-acting elements within the* AtPGM *promoter*

Because we discovered that the *AtPGM* promoter displays responsiveness to the different conditions tested, we analyzed this promoter sequence for known *cis*-acting elements by means of the PLACE (plant *cis*-acting elements) database (Higo *et al.*, 1998). As shown in Figure 4, we found that the *AtPGM* promoter contains the GmSAUR sequence, which has been shown to be the auxin-responsive element in the soybean *SAUR* (small auxin-up RNA) 15*A* gene promoter (Xu *et al.*, 1997). Furthermore, the *AtPGM* promoter contains an ABA-responsive element (ABRE) (Busk and Pagès, 1998) and a sugar response element (Hwang *et al.*, 1998). Potentially

explaining the *AtPGM* promoter nematode responsiveness, we discovered the CAATTG nematode box, which has been shown to bind specifically to nuclear proteins from root-knot nematode-induced galls from tomato roots (Escobar *et al.*, 1999). Interestingly, we also discovered in the *AtPGM* promoter a sequence with 90% identity to the mitosis-specific activator (MSA) core consensus binding site [(T/C)C(T/C)AACGG(T/C)(T/C)], which has been shown to be necessary and sufficient for mitosis-specific promoter activation (Ito *et al.*, 1998). Although intriguing, the significance and functionality of these *AtPGM cis* elements have to be established experimentally.

Analyses of the auxin-responsive AUX1 *promoter in nematode-infected plants*

The *AtPGM* promoter is auxin-responsive as well as active in developing feeding structures of cyst and root-knot nematodes. Nematode feeding cell formation has been reported to be accompanied by local changes in auxin signal transduction. It has been postulated that nematodes locally manipulate auxin concentrations by perturbing polar auxin transport (Hutangura *et al.*, 1999; Goverse *et al.*, 2000b). Additionally, a recent study postulated that nematode secretions actively alter cell metabolism resulting in lowered auxin biosynthesis after an initial auxin increase (Doyle and Lambert, 2003). To further investigate the role of auxin in the plant-nematode interactions and to assess whether the observed *AtPGM* changes in nematode feeding structures may be due to auxin influences, we assessed the characteristics of the auxin-responsive promoter of *AUX1*, a gene coding for a polar auxin transport protein, upon nematode infection. Prior to these experiments, we confirmed the meristem-specific expression of the *AUX1* promoter in uninfected plants previously reported by Marchant *et al.* (1999) under our conditions and established the induction of the *AUX1* promoter by exogenously applied auxin (Figure 8A and B, respectively). Transgenic *A. thaliana* plants expressing an *AUX1* promoter*::GUS* construct (Marchant *et al.*, 1999) were analyzed for GUS expression at 3, 7, and 14 dai with *H. schachtii* or *M. incognita*. A pattern of promoter activation very similar to that of the *AtPGM* promoter was found, particularly inside the cyst nematode and root-knot nematode feeding structures. As shown in Figure 8, GUS activity was induced in *H. schachtii*-induced syncytia at 3 dai (Figure 8C), 7 dai (Figure 8D), and 14 dai (Figure 8E). Similarly, *M. incognita*-induced galls demonstrated increased GUS activity at 3 dai (Figure 8F), 7 dai (Figure 8G), and 14 dai (Figure 8H).

Discussion

Phosphoglycerate mutase (PGM) (EC 5.4.2.1) is a key enzyme catalyzing the reversible interconversion of 3-phosphoglycerate and 2-phosphoglycerate during sugar metabolism in the glycolysis pathway (reviewed by Jedrzejas, 2000). There are two types of PGM: one that requires 2,3-bisphosphoglycerate as a cofactor (cofactor-dependent; PGM-d) and the other that does not (cofactor-independent; PGM-i). Although both the PGM-d and PGM-i types catalyze the same reaction, there is no sequence similarity between these two enzymes. In fact, sequence analysis of PGM-d proteins has shown that these enzymes exhibit strong sequence similarity to bisphosphoglycerate mutase (bPGM, EC 5.4.2.4), which primarily catalyzes the interconversion of 1,3-bisphosphoglycerate and 2,3-bisphosphoglycerate (reviewed by Jedrzejas, 2000). bPGM also is a component of the glycolysis pathway. Interestingly, PGM-d and bPGM have the ability to catalyze each other's preferred reactions albeit at slower rates. Similar catalytic functions and significant sequence conservation of PGM-d and bPGM are the reasons why these two enzymes form a group of isoenzymes referred to as phosphoglycerate/bisphosphoglycerate mutase (PGM/bPGM; reviewed by Jedrzejas, 2000).

We have identified the nematode-responsive genes *GmPGM* in soybean and *AtPGM* in *Arabidopsis*. Our sequence analysis results indicated that the predicted proteins GmPGM and AtPGM belong to the PGM-d/bPGM but not to the PGM-i type of PGM. PGM-d/bPGM members have been identified in animals, fungi, eubacteria, and archaea (Jedrzejas, 2000; van der Oost *et al.*, 2002). We found that PGM-d/bPGM sequences generally are present in plant EST collections (e.g., rice, GenBank accssion number CA767207; bean, CA896652; maize, TIGR maize gene index TC139242, TC139243; wheat, TC101623), and that many of these ESTs have been isolated from stressed plants following nutrient deficiency, drought, fungal infection, and hormone treatments. However, published reports from plants are only available for the *PGM-i* type (Carreras *et al.*, 1982; Smith and Hass, 1985; Botha and Dennis,

1986; Grana *et al.*, 1989, 1992, 1995; Huang *et al.*, 1993; Wang *et al.*, 1996) including the reported up-regulation of such a tomato gene upon viral infection (Itaya *et al.*, 2002). The data on PGM-d/bPGM-type genes presented here provide the first insight into the characteristics and putative functions of these genes in plants.

Of most interest to us is the fact that the soybean and *Arabidopsis PGM* genes identified here are nematode-responsive, particularly in the nematode-induced feeding structures, which makes their study beneficial to an understanding of plant-nematode interactions. We determined that *AtPGM* is up-regulated in the tissues of developing and mature feeding structures of cyst and root-knot nematodes in *Arabidopsis*. It is likely that a similar pattern of expression exists for *GmPGM* in nematode-infected soybean roots, although this has not been assessed. *AtPGM* is up-regulated in both types of developing nematode feeding structures as early as 3 dai, and this activity increased with time, reflecting the expanding size and activity of these plant cells. Previously, Favery *et al.* (1998) showed that the *Arabidopsis RPE* gene coding for ribulose-phosphate 3-epimerase (EC 5.1.3.1), an enzyme also involved in sugar metabolism (pentose phosphate pathway), is specifically up-regulated in the feeding cells induced by *H. schachtii* or *M. incognita* up to 15 dai. Additionally, a recent study by Juergensen *et al.* (2003) showed that an *Arabidopsis* sugar transporter gene (*AtSUC2*) is expressed in syncytia. Taken together, these findings suggest, as expected, that sugar intake into nematode feeding cells is elevated followed by an increased rate of metabolism through glycolysis and pentose phosphate pathways.

Besides a mere increase in sugar metabolism through elevated glycolysis and pentose phosphate pathway throughput (as suggested by *AtPGM* and *RPE* up-regulation, respectively), there is at least one other possible scenario that would give these observations importance in the plant-nematode interaction. It recently has been proposed that the root-knot nematode *M. javanica* secretes a chorismate mutase isoform into the cytoplasm of developing feeding cells (Doyle and Lambert, 2003). This enzyme also appears to be secreted by cyst nematodes (Popeijus *et al.*, 2000; Bekal *et al.*, 2003; Gao *et al.*, 2003). Chorismate mutase is a key branchpoint of the shikimate pathway in that it is responsible for the conversion of chorismate to prephenate. Chorismate and prephenate are precursors of different aromatic amino acids, which in turn give rise to a number of molecules with known functions in plant-pathogen interactions, including auxin, salicylic acid, and phytoalexins. Most interestingly for this paper, the synthesis of chorismate in the shikimate pathway begins with the condensation of the products of glycolysis and the pentose phosphate pathway (phospho*enol*pyruvate (PEP) and erythrose 4-phosphate, respectively), i.e., the pathways for which key enzyme genes are up-regulated in nematode feeding structures. Therefore, it is tempting to hypothesize that the observed up-regulation of *AtPGM* and *RPE* genes not only serve to meet increased energy demands but very specifically also provide the necessary substrate for the shikimate pathway.

Another noteworthy observation made in this study is that during normal plant development, the *AtPGM* promoter directs GUS activity specifically to the root and shoot apical meristems. Similar observations have been made for *RPE*, cell-cycle-related genes and several other genes (Goddijin *et al.*, 1993; Niebel *et al.*, 1996; Barthels *et al.*, 1998; Favery *et al.*, 1998, 2002; de Almeida Engler *et al.*, 1999; Goverse *et al.*, 2000a; Koltai *et al.*, 2001). Obviously, nematode-feeding cells and meristematic cells share a cytoplasmically dense and metabolically active state as well as cell cycle activity (Golinowski *et al.*, 1996, 1997; Gheysen *et al.*, 1997; de Almeida Engler *et al.*, 1999), which probably accounts for the observed co-regulation in both cell types. The observed expression of *AtPGM* in root meristems and the developing nematode feeding structures further demonstrates that the physiology of these plant cell types share common steps.

This co-regulation in meristems and feeding sites is further emphasized by the specific regulatory functions of sucrose, oryzalin, ABA, and auxin on *AtPGM* expression. It has been shown that sugars can provide positional information to the cell cycle machinery and developmental programs, and that cell division and differentiation are, in fact, ascribed to changes in metabolic activity (reviewed by Rolland *et al.*, 2002). For example, in *Vicia faba* embryos, sugar gradients have been reported to correlate spatially with mitotic activity (Borisjuk *et al.*, 1998). *Arabidopsis* mitotic cell cycle gene expression is also regulated differentially by sugars (Riou-Khamlichi *et al.*, 2000). Interestingly, analyses of tomato meristems revealed spatially regulated sugar metabolism within the meristem and suggested the involvement of sugar metabolism in organogenesis (Pien *et al.*, 2001). Therefore, the sucrose-responsive regulation of *AtPGM* could be one of the stimuli responsible for expression in both meristems and nematode feeding structures. A poten-

tial role of AtPGM in cell divisions would also explain why *AtPGM* expression is induced by oryzalin because oryzalin-induced gene expression is regarded as diagnostic of genes with functions during mitosis (Ferreira *et al.*, 1994a, b).

A similar conclusion can be reached by our finding that ABA represses *AtPGM* expression. Repressed expression of genes related to meristematic activity by exogenous ABA has been shown in both *Arabidopsis* and tobacco (Hemerly *et al.*, 1993), and an inhibitory effect of cell division in roots by ABA also has been described (Phillips, 1971; Newton, 1977). Additionally, it has been shown that the *Arabidopsis pyK20* (an up-regulated nematode-responsive gene) is repressed by ABA (Puzio *et al.*, 2000). Nevertheless, the role of ABA in plant-nematode interactions has not yet been defined and remains to be investigated.

The fact that *AtPGM* expression is induced by auxin not only ties this gene to cell-cycle regulation but also may further our understanding of the role that auxin plays in feeding site formation. At the cellular level, auxin acts as a signal for cell division, extension, and differentiation (Davis, 1995). Several studies have shown that auxin signaling is essential in feeding cell formation by cyst and root-knot nematodes. One line of work shows that cyst and root-knot nematodes induce a local accumulation of auxin in their respective feeding structures presumably by perturbing polar auxin transport through inhibition of auxin efflux carriers (Hutangura *et al.*, 1999; Goverse *et al.*, 2000b). On the other hand, a recent study by Doyle and Lambert (2003) proposed that nematodes actually lower auxin biosynthesis after this early auxin increase. We assessed the auxin-responsive *AUX1* promoter in GUS fusion lines in this study for two reasons: (1) to assess whether the observed *AtPGM* expression pattern mirrors the expression of another auxin-responsive gene and (2) to assess whether an auxin influx carrier is up-regulated in nematode feeding structures. We showed that the *AUX1* promoter, in addition to its auxin induction, directs GUS expression to the developing feeding sites of cyst and root-knot nematodes along with root tips, i.e., in a manner very similar to *AtPGM*. Since both these genes are regulated by auxin, these observations suggest that auxin may at least in part be responsible for the observed expression patterns of both genes. Furthermore, *AUX1* up-regulation in nematode feeding sites is in line with the data of Hutangura *et al.* (1998) and Goverse *et al.* (2000b) that a localized auxin increase can be observed in the feeding structures of both root-knot and cyst nematodes. Taking these results one step further, we propose that an increase in auxin may be accomplished through an increased auxin influx in addition to an inhibition of auxin efflux, as previously suggested. On the other hand, our data are not fully in agreement with Hutangura *et al.* (1999) in that *AUX1*- as well as *AtPGM*-directed GUS expression persisted in the feeding structures longer than the 92 h period after inoculation with root-knot nematodes reported by these authors. We also did not observe a down-regulation of the *AtPGM* and *AUX1* promoters as one would expect from a decrease in auxin concentration in the nematode feeding cells postulated by Doyle and Lambert (2003). However, since we have shown several regulators of *AtPGM* expression, a decrease in auxin concentration may have been masked by other stimuli.

In conclusion, the present study identified *PGM* genes in soybean and *Arabidopsis* that are associated with meristems and the development of feeding structures of both cyst and root-knot nematodes. Further research of these genes promises to reveal intriguing insights into the molecular mechanisms involved in the complex interactions between plants and sedentary nematodes and also the roles of plant *PGM* genes in general.

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